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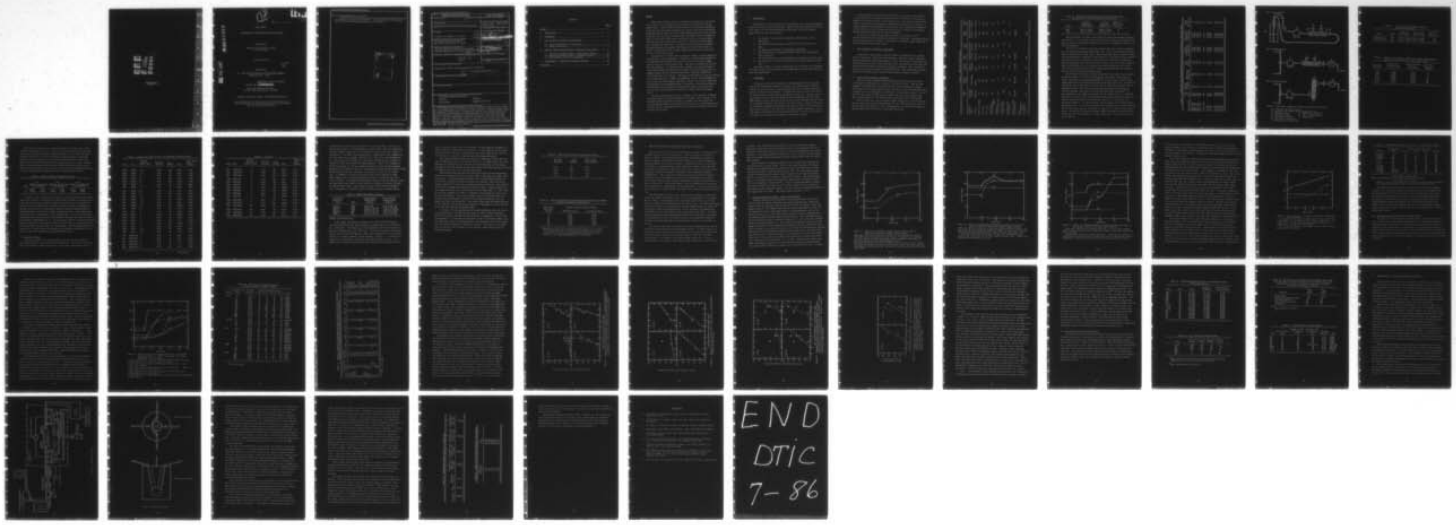

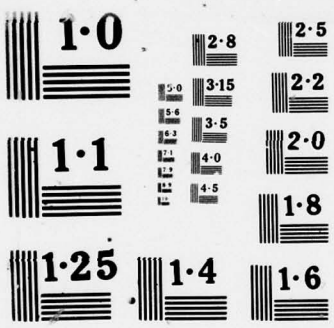

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FINAL REPORT

DEVELOPMENT OF AN ON-LINE BIOLOGICAL DETECTOR

Presented by

Kenneth C. Ehrlich, Elias Klein
and J.K. Smith

October 30, 1977

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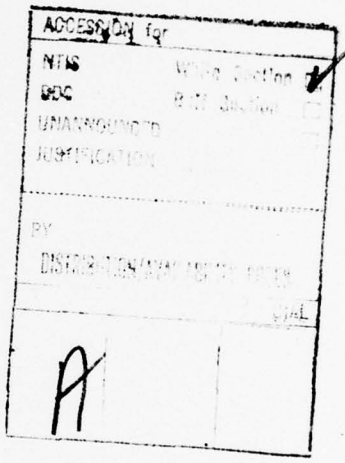
20. percent inhibition of cell growth.

Adaptation of the perfusion system for water toxicity screening and for use outside a warm room was explored. A prototype detector flow system, which utilizes once-through perfusion, is described.



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Artificial capillary perfusion culture of mammalian cells is being tested for its effectiveness as a detector of toxic substances. Using change in cellular oxygen utilization rate as a measure of toxicity, the effects on L929 cells of o-toluidine, hydroquinone, 2,3-dimethylphenol, 2,4-dinitrophenylhydrazine, potassium cyanide, N,N-diethyl-m-toluamide and sodium iodoacetate have been studied. The toxic substance is added to the medium stream, and after 60-90 minutes' exposure of the cells, curves of log dose versus percent inhibition of oxygen utilization are comparable to those obtained for log dose versus --(over)

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CONTENTS

SUMMARY

Oxygen utilization by cells on artificial capillaries has been found to be sensitive to a variety of toxic substances incorporated into the medium stream. BHK or L929 cells grown in the extracapillary compartment of 80-150 fiber artificial capillary cell culture units use 0.073 ± 0.025 μmoles/min glucose and 0.76 ± 0.26 μl/min oxygen and excrete 0.078 ± 0.038 μmoles/min lactic acid by 7-10 days after initiation of the cell cultures. Except at flow rates ≤0.05 ml/min, metabolic rate was not significantly affected by change in flow rate or by use of different medium perfusion modes (through the fiber bore or through the extracapillary compartment). The cell population (estimated from glucose and oxygen utilization as 20×10^6 cells/unit) does not increase significantly when the serum (calf) content of the medium is ≤1%.

Incorporation of toxins such as potassium cyanide, sodium iodoacetate, hydroquinone, 2,3-dimethylphenol, o-toluidine, 2,4-dinitrophenylhydrazine, and N,N-diethyl-m-toluamide into the nutrient stream inhibited oxygen utilization by the cells within 90 min. The amount of inhibition was dose and time dependent. At 30-90 min after treatment, the concentrations of toxins which caused 50% inhibition of oxygen utilization (EC_{50}) were only slightly higher than the concentrations which cause 50% inhibition of cell growth after 72 hr (LC_{50}). The EC_{50} concentrations were for o-toluidine, 32 ppm; hydroquinone, 3.2 ppm; dimethylphenol, 36 ppm; dinitrophenylhydrazine, 22 ppm; and N,N-diethyl-m-toluamide, 250 ppm. In certain cases (with hydroquinone, dinitrophenylhydrazine, o-toluidine, and potassium cyanide), the inhibition was reversed by perfusion with nontoxic medium when the initial dose was sufficiently low and the treatment time sufficiently short.

Based on oxygen utilization as a measure of cell function, a compact prototype monitor for water potability is proposed. Some features of the proposed prototype have been tested; for example, the requirement that the cell growth chamber be maintained at 37° by use of a small heating bath and that the water and medium stream can be mixed satisfactorily to provide nutrient to the cells.

1. INTRODUCTION

The objective of this program is the development of an on-line biological detector for water toxicity monitoring. Before designing a prototype detector, toxic effects on cell metabolism must be understood. With this purpose, studies have been made to determine:

1. The length of time artificial capillary cell cultures can be maintained;
2. The stability of the oxygen utilization rate as a function of time;
3. The medium requirements for maintenance perfusion;
4. The feasibility of "through-the-shell" rather than "through-the-bore" perfusion;
5. The reproducibility of cell establishment on the culture units; and
6. The effects of selected toxic materials on cellular oxygen utilization.

From these studies, the basic requirements for an on-line, fast-response biological detector using mammalian cell metabolism as the toxicity indicator have been established.

2. BACKGROUND

During the first year of research in the biological detector program, some of the difficulties involved in routine cell culture using artificial capillaries were overcome, and the perfusion system for rapidly monitoring cellular metabolism was developed. Attempts were made to measure the effect of toxins on lactic acid excretion and glucose utilization. However, these measurements were not sensitive enough to meet the objectives of the Army MUST program; namely, that the toxicity detector have a response time of 15-30 min and permit continuous monitoring of the water stream. Oxygen utilization is a metabolic parameter of mammalian cells which is known to be sensitive to many types of toxins.[1] This parameter satisfies program requirements, since measurement can be made with an oxygen electrode and since the cell number on the units can be adjusted to heighten the observable response.

Using cyanide, iodoacetate, and o-toluidine as toxins, it was found that a rapid inhibition of oxygen utilization could be elicited by incorporating appropriate amounts of toxin in the medium stream. The response using through-the-bore perfusion (flow through the capillaries only) was slower than that using through-the-shell perfusion (flow through the extracapillary compartment of the culture unit) with iodoacetate and cyanide; no response was elicited during perfusion through-the-bore with o-toluidine.

In the first section of this report, aspects of our work on cell culture using artificial capillary perfusion are summarized. Subsequent sections of the report detail the studies of the effects on cellular oxygen utilization of toxins added to the medium stream.

3. CELL CULTURE ON ARTIFICIAL CAPILLARIES

In the first annual report (July 31, 1976), the methods used to culture cells on artificial capillaries were described. Since the report, additional data concerning the rapid establishment of cells on artificial capillaries have been obtained; new culture units have been used; and comparisons have been made between different modes of artificial capillary perfusion. The results from first-year and current studies are discussed below. The dimensions and properties of the capillary units used for these studies are given in Table 1.

3.1 Lactic Acid and Glucose Metabolism

Only one report has been made describing glucose utilization by cells on artificial capillaries[2] as a function of time in culture. This and other studies[3,4] indicate that the transfer of cells from a traditional culture environment to artificial capillary units requires a period of adaptation before a substantial cell population is established. In one study, however,[5] a claim was made that growth to 10^9 cells per culture unit can occur within 9 days after inoculation of 5×10^5 virus-transformed hamster embryo fibroblasts.

We have found that, on the average, within 2 weeks after initial planting, the cell population reaches approximately $21 \pm 8 \times 10^6$ cells/culture unit for BHK and L929 cells (Table 2). This cell population was calculated assuming an average glucose utilization of 5 μmoles/10^6 cells/day.[6]

TABLE 1. CAPILLARY CULTURE UNITS

	GSRI 75	Amicon 3S 100	Amicon 3 x 50	GSRI 150	GSRI 300	GSRI 500	GSRI 80
Capillary type	Cellulose Acetate	Polysulfone	Acrylic Copolymer	Cellulose Acetate	Cellulose Acetate	Cellulose Acetate	Cellulose Acetate
Potting	Silastic	Epoxy	Epoxy	Silastic	Urethane	Urethane	Urethane
Number of Fibers	75	150	150	150	300	500	80
I.D. (μm)	275	200	200	109	250	250	250
O.D. (μm)	475	350	350	200	350	350	350
Total area, fiber surface (cm^2)	48	94	94	57	307	511	52
Effective fiber length (cm)	6.0	5.7	5.7	6.0	9.3	9.3	5.9
Intracapillary volume (ml)	.269	.27	.27	.09	.86	1.45	.23
Extracapillary volume (ml)	0.8	2.6	2.6	0.6	14	12	2.1
Molecular weight permeability	>64,000	100,000	50,000	>64,000		>64,000	
Water permeability L_p $(\frac{cm}{atm\ sec})$ x 10^5	747	240	80	970		1125	

TABLE 2. AVERAGE GLUCOSE CONSUMPTION AND LACTATE EXCRETION BY BHK AND L929 CELLS ON ARTIFICIAL CAPILLARIES

Cells	Glucose Consumption (μmoles/min)	Lactate Excretion (μmoles/min)	Number of Experiments Averaged
BHK	0.072 ± 0.020	0.086 ± 0.034	9
L929	0.075 ± 0.037	0.065 ± 0.047	5

Table 3 presents the cell culture data from which the averages in Table 2 were derived. It is evident from the data neither planting with different cell numbers nor use of different reservoir volumes has a significant effect on the cell establishment.

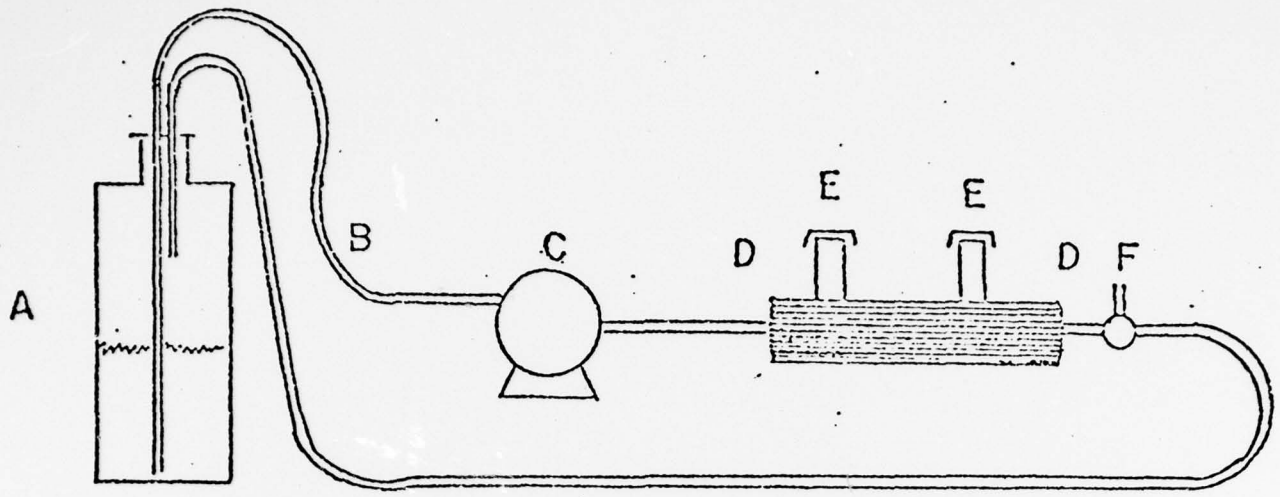
The results in Tables 2 and 3 were derived from cell cultures maintained by a recirculating perfusion system (Fig. 1a). Use of once-through perfusion either through-the-bore (Fig. 1b) or through-the-shell (Fig. 1c) gives somewhat lower average glucose utilization rates (Table 4). These measurements, however, were made on cultures perfused at a flow rate of 0.05 ml/min, at which the glucose utilization rate and lactic acid excretion rate were found to decrease significantly (Table 5). No difference was found between the lactic acid excretion or glucose utilization by cultures perfused through the shell and that by cultures perfused through the bore.

The effect of flow rate on glucose utilization and lactic acid excretion is shown in Table 5. As flow rate decreases, the glucose utilization and lactic acid excretion increase, presumably due to the Pasteur effect,[7] since oxygen depletion of the medium must occur as the flow rate slows. The glucose utilization rate of the cells at a flow rate of 0.60 ml/min indicates that a population of 28×10^6 cells was present on this culture unit (using an estimated glucose utilization rate of 0.0035 μmoles/10^6 cells/min).[6] At this population, the oxygen utilization rate is estimated to be 1.4 μl/min.[7] From this utilization rate, the estimated outlet oxygen tension of the medium stream can be calculated at the different flow rates. These values are included in Table 5. At low flow rates, all the oxygen in the medium would probably be used. As the medium becomes more anaerobic due to oxygen depletion by the cells, more glucose is utilized to support the cellular energy requirements. At very slow flow rates, however, glucose metabolism appears to slow to one-half the value exhibited at higher flow rates. The reason for this is as yet unclear.

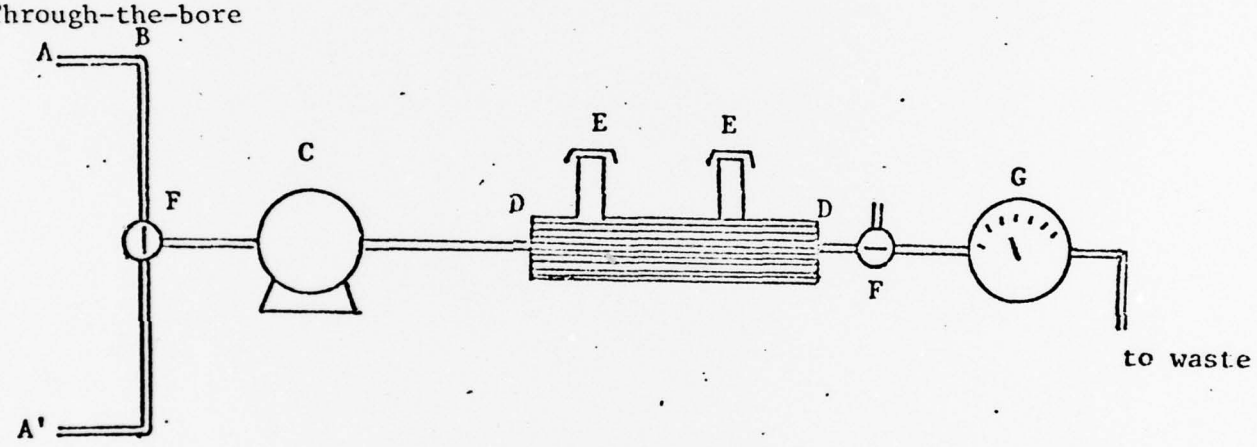
TABLE 3. LACTIC ACID AND GLUCOSE METABOLISM BY CELLS ON ARTIFICIAL CAPILLARY UNITS

Type of Unit	Type of Cells	Amount Planted ($\times 10^{-6}$)	Reservoir Volume (ml)	Flow Rate (ml/min)	Glucose Utilization (μ moles/min)	Lactic Acid Excretion (μ moles/min)	Days After Initiation
GSRI (75)	BHK	21	100	2.0	0.067	0.167	9
GSRI (75)	BHK	12	50	2.0	0.062	0.094	9
GSRI (75)	BHK	14	25	5.0	0.135	0.132	10
GSRI (75)	BHK	14	25	5.0	0.089	0.091	10
GSRI (75)	BHK	14	25	5.0	0.049	0.033	10
GSRI (150)	BHK	21	50	2.0	0.087	0.076	14
3S 100	BHK	21	75	3.0	0.046	0.060	10
3S 100	BHK	21	75	3.0	0.055	0.043	10
3S 100	BHK	21	75	3.0	0.062	0.075	10
3S 100	L929	21	75	3.0	0.044	0.039	10
3S 100	L929	21	75	3.0	0.055	0.046	10
3S 100	L929	21	75	3.0	0.063	0.026	10
3S 100	L929	21	75	3.0	0.045	0.032	10
3S 100	L929	20	50	8.5	0.167	0.183	9

(a) Recirculating



(b) Through-the-bore



(c) Through-the-Shell

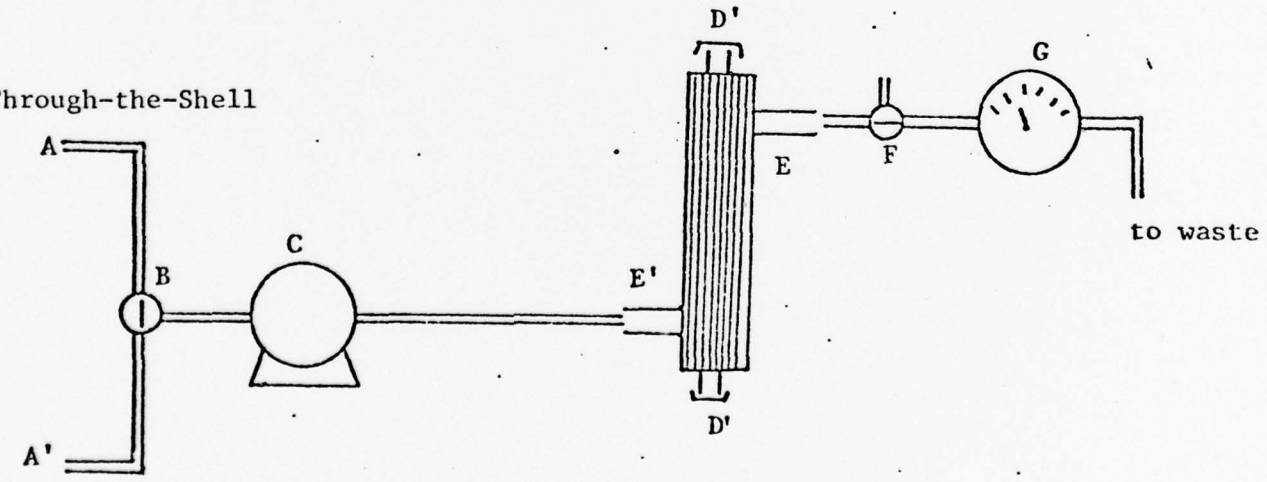


Figure 1. Perfusion schemes for artificial capillary cell culture

A - reservoir for tissue culture
A'- reservoir for tissue culture medium plus toxin
B - Silastic tubing
C - peristaltic pump
D - male Luer connectors
D'- male Luer to closed end
E - female Luer to closed end
E'- female Luer connectors
F - Luer - 3-way stopcock
G - oxygen meter

TABLE 4. LACTIC ACID AND GLUCOSE METABOLISM: ONCE-THROUGH-PERFUSION

Mode	Cells	Glucose (μmoles/min)	Lactic Acid (μmoles/min)	Number of Experiments
Through Bore	BHK	0.045 ± 0.001	0.074 ± 0.007	(3)
Through Shell	BHK	0.043 ± 0.006	0.097 ± 0.009	(3)

TABLE 5. EFFECT OF FLOW RATE ON GLUCOSE AND LACTIC ACID METABOLISM (BHK Cells on GSRI 150 Unit; Flow Through Bore)

Flow Rate (ml/min)	Lactic Acid Excretion Rate (μ moles/min)	Glucose Utilization Rate (μ moles/min)	Estimated Outlet pO_2 (mmHg)
0.08	0.088	0.068	0
0.18	0.165	0.145	0
0.21	0.165	0.140	0
0.32	0.149	0.128	26
0.45	0.123	0.106	66
0.60	0.115	0.099	86

The effects of medium serum content on glucose utilization and lactate excretion are shown in Table 6. Using a recirculating perfusion system, BHK cells were initiated on parallel capillary culture units in which the medium contained either 5%, 1% or 0.1% calf serum. Based on glucose utilization results, the cell population by day 6on units perfused with 5% calf serum medium was more than double that of units perfused with 0.1% serum. Furthermore, the units perfused with 0.1% serum showed no growth from days 4 to 6, whereas the units perfused with either 1% or 5% serum showed significant growth. A slower initial growth is apparent with 1% serum-initiated cultures.

TABLE 6. EFFECT OF SERUM ON METABOLIC ACTIVITY

	5%		1.0%		0.1%	
Day	Glucose	Lactic Acid	Glucose	Lactic Acid	Glucose	Lactic Acid
	(μ moles/min)		(μ moles/min)		(μ moles/min)	
4	0.081	0.114	0.045	0.064	0.043	0.065
6	0.097	0.146	0.062	0.062	0.040	0.073

In several cases, capillary cell cultures have been maintained for periods longer than 2 weeks. Our results (see Annual Report, 1976) are in basic agreement with those of other workers;[5] namely, that cell cultures on artificial capillaries can be maintained for months provided the medium is changed frequently, the flow rate is sufficiently rapid to prevent prolonged periods of oxygen depletion, and the unit is kept contamination free. The current study has made several contributions to these observations: (1) Cells on artificial capillaries can be grown outside a CO_2 incubator provided the medium is buffered by addition of 20mM HEPES, pH 7.3. (2) A plateau in growth is reached relatively quickly (within 1-2 weeks) when the medium contains 1% calf serum; use of 0.1% calf serum medium for maintenance is also possible. (3) Prolonged perfusion (at least up to 2 weeks) using once-through flow is possible provided the flow rate is not so slow that inordinate nutrient depletion results.

3.2 Oxygen Metabolism

Some of the latter data discussed above were derived from studies of oxygen utilization by cells on artificial capillaries. The oxygen utilization data from a number of different types of culture units planted with either BHK

TABLE 7. OXYGEN UTILIZATION BY CELLS ON ARTIFICIAL CAPILLARY UNITS

Cells	Unit	Initial Planting (cells x 10^{-6})	Flow Rate (ml/min)	$\Delta_p O_2$ (mm Hg)	Mode	Utilization Rate (µl/min)
L929	3S100	5.6	0.46	130	bore	2.21
L929	3S100	7.5	0.21	55	bore	0.42
L929	3S100	13	0.21	78	bore	0.60
L929	3S100	11	0.27	100	bore	1.00
L929	3S100	15	0.31	110	shell	1.26
L929	3S100	6	0.16	48	bore	0.28
L929	3S100	5.5	0.31	75	shell	0.86
BHK	3S100	30	0.25	70	bore	0.65
BHK	3S100	30	0.25	90	bore	0.83
BHK	3S100	7.5	0.31	115	bore	1.32
BHK	3S100	7.5	0.31	78	shell	0.89
BHK	3S100	7.5	0.29	59	bore	0.63
BHK	3S100	7.5	0.29	85	shell	0.91
BHK	3S100	30	0.25	112	shell	1.04
L929	3x50	7	0.17	56	shell	0.42
L929	3x50	7	0.17	58	shell	0.35
L929	3x50	7	0.17	54	shell	0.36
L929	3x50	7	0.24	90	shell	0.48
L929	3x50	7	0.24	127	shell	1.12
L929	3x50	5	0.26	105	shell	1.01
L929	3x50	6.5	0.29	45	bore	0.48
L929	3x50	6.5	0.29	70	shell	0.75
BHK	GSRI(150)	7	0.18	95	bore	0.63
BHK	GSRI(150)	7	0.29	34	bore	0.36
BHK	GSRI(150)	7	0.16	32	bore	0.19
BHK	GSRI(150)	13	0.35	70	bore	0.90
BHK	GSRI(150)	13	0.25	115	bore	1.06

(continued)

TABLE 7. Continued

Cells	Unit	Initial Planting (cells x 10^{-6})	Flow Rate (ml/min)	$\Delta_P O_2$ (mm Hg)	Mode	Utilization Rate (µl/min)
BHK	GSRI(150)	9	0.25	119	bore	1.10
BHK	GSRI(150)	9	0.25	85	shell	0.79
BHK	GSRI(150)	9	0.25	115	shell	1.06
L929	GSRI(80)	7	0.25	102	bore	0.94
L929	GSRI(80)	7	0.24	88	bore	0.78
L929	GSRI(80)	7	0.24	40	shell	0.35
L929	GSRI(80)	7	0.22	50	shell	0.41
L929	GSRI(80)	7	0.21	110	shell	0.85
L929	GSRI(80)	7	0.21	52	shell	0.40
L929	GSRI(80)	7	0.31	70	shell	0.80
L929	GSRI(300)	7	0.51	127	bore	2.40
L929	GSRI(300)	7	0.20	131	shell	0.97
L929	GSRI(300)	40	0.34	112	shell	1.41
L929	GSRI(500)	21	0.17	92	bore	0.58
L929	GSRI(500)	21	0.30	133	bore	1.48
L929	GSRI(500)	21	0.31	114	bore	1.31

or L929 cells at different cell densities are given in Table 7; the data are summarized in Table 8. The measurement of oxygen utilization rate was made on cultures allowed to grow for 1 to 2 weeks after initial planting. Except for the Amicon 3 x 50 culture units (which have a significantly lower permeability than the other units; $L_p(H_2O)$ = 80 cm/atm sec, whereas for the GSRI-80 units, L_p = 1125 cm/atm sec, see Table 1), the oxygen utilization rates reached an average of 0.76 ± 0.26 µl/min (29 determinations). There is no significant difference between the average oxygen utilization of units perfused through-the-bore (0.79 µl/min) and that of units perfused through-the-shell (0.72 µl/min). Either mode of perfusion, therefore, can sustain the cells on the culture unit. In addition, no loss of cells due to the flow of medium over them (which might occur in through-the-shell perfusion), is observed after this time in culture. The oxygen utilization data indicate that the different units are comparable with respect to their ability to support cell growth (at least for BHK and L929 cells). These results confirm those of Wolf and Munkelt[2] from a comparison of cellulose acetate and Amicon acrylic copolymer fibers.

TABLE 8. OXYGEN UTILIZATION (Summary)

Artificial Capillary Unit	Cells	Oxygen Uptake Through-Bore (µl/min)	Oxygen Uptake Through-Shell (µl/min)
3 X 50	L929	0.48 (1)[a]	0.59 ± 0.22 (7)
GSRI-80	L929	0.86 ± 0.08 (2)	0.56 ± 0.21 (5)
3S100	L929	0.90 ± 0.56 (5)	1.06 (± 0.20) (2)
GSRI-150	BHK	0.71 ± 0.31 (6)	0.92 ± 0.14 (2)
3S100	BHK	0.85 ± 0.23 (4)	0.95 ± 0.06 (3)

[a]Number of determinations.

If the oxygen consumption is assumed to be 0.047 µl/10^6 cells/min,[7] the cell population on the units reaches an average value of 17 x 10^6 cells per culture unit. This number is in close agreement with that obtained from glucose utilization data, 21 x 10^6 cells. We assume, therefore, that on the small culture units of 150 fibers or less, such a population can be achieved within 1 or 2 weeks following initial planting. Furthermore, a greater cell population is undesirable, since more cells would demand a greater amount of oxygen from the medium, which could be provided only by increasing the flow

rate. At a utilization rate of 0.76 µl/min, the ΔpO_2 would be 86 mmHg for a unit perfused at a flow rate of 0.24 m/1min. At this ΔpO_2 the outlet oxygen tension would be ∿60 mmHg, a value which would ensure that all the cells on the culture unit receive an adequate supply of oxygenated medium.

The oxygen utilization rate did not show a significant change with flow rates varying form 0.24 to 1.24 ml/min (Table 9). At slower flow rates (0.11 ml/min), the utilization rate could not be determined since all the oxygen was depleted from the medium; at flow rates higher than those listed in Table 9, the ΔpO_2 was too small to be measured accurately.

These studies indicate that both through-the-shell and through-the-bore perfusion can maintain cells on the units without affecting the oxygen utilization rate. As mentioned above (Section 3.1), once-through perfusion can be used for prolonged artificial capillary cell culture. When the culture medium contains 0.1% calf serum (as with the glucose and lactic acid studies), no increase in oxygen utilization was found in 2 days; a slight increase in the oxygen utilization rate occurred with 1% calf serum (Fig. 2). This study has now been extended to 2 weeks of continuous once-through perfusion using 1% calf serum medium. Even with the higher serum levels (under which cell division is not prevented), a plateau in the oxygen utilization rate was found with L929 cells (Table 10). The average final ΔpO_2 (114 mmHg) obtained at the flow rate of 0.24 ml/min may represent a limitation in cell population due to the unit geometry and medium supply.

In summary, the average glucose and oxygen utilization rates and lactic acid excretion rates for BHK and L929 cells are 0.073 ± 0.025 µmoles/min, 0.76 ± 0.26 µl/min, and 0.078 ± 0.038 µmoles/min, respectively. These rates corresponded to an estimated cell population of 20×10^6 cells/capillary unit. The utilization rates for through-the-bore and through-the-shell once-through flow are, within experimental variation, the same. The limit to the usable life of such units has not been set, but exceeds 2 weeks using any of the perfusion methods.

TABLE 9. EFFECT OF FLOW RATE ON OXYGEN UTILIZATION

Flow Rate (ml/min)	$\Delta_p O_2$ (mm Hg)	Utilization Rate (µl/min)
0.11	141	0.57
0.24	118	1.04
0.50	74	1.37
1.24	22	1.01

TABLE 10. EFFECT OF PROLONGED ONCE-THROUGH FLOW ON OXYGEN UTILIZATION BY CELLS ON ARTIFICIAL CAPILLARIES[a]

Time after Initiation (days)	Oxygen Utilization Rate (µl/min)	
	Through-the-Bore	Through-the-Shell
7	0.74	0.69
9	0.77	0.72
11	0.78	0.77
14	0.78	0.79
18	0.78	0.80
21	0.78	0.80

[a]GSRI-80 capillary units planted with L929 cells; cell cultures were initiated using a recirculating perfusion mode and maintained for 7 days prior to beginning once-through perfusion; flow rate for once-through-perfusion, 0.24 ml/min.

4. TOXICITY STUDIES USING ARTIFICIAL CAPILLARY CELL CULTURE

We have tested the toxicity of 8 substances on oxygen utilization by BHK or L929 cells on artificial capillaries. These substances include three phenols: phenol, 2,3-dimethylphenol (2,3-DMP), and hydroquinone (HQ); an insect repellant, N,N-diethyl-m-toluamide (DET); a dye, 2,4-dinitrophenylhydrazine (DNPH); an aniline derivative, o-toluidine (o-tol); and two metabolic poisons: potassium cyanide, which inhibits the final step in electron transport, and iodoacetate, which can react with a number of proteins, and in particular, inhibits glycolysis by binding to glyceraldehyde phosphate dehydrogenase. These toxins were incorporated into the perfusion medium at a particular dose, and perfusion (using a once-through mode) was continued for at least 120 min unless complete inhibition of oxygen utilization occurred prior to that time. In some experiments, the toxic response was reversed by the introduction of fresh medium not containing toxin. In all cases to date, the toxic response was manifested by a decrease in the cellular oxygen utilization rate.

The introduction of toxin to the cells on the capillaries has been performed in a number of different ways. In our first experiments (see Annual Report, 1976), a syringe pump was used to perfuse medium with toxin through either the shell or the capillaries of the culture unit. For these experiments, the flow of medium to the culture unit was interrupted during the change from toxin-free to toxin-containing medium. In all experiments performed since July 1976, no such interruption of flow to the unit was necessary. In these latter studies, perfusion was maintained with a peristaltic pump. The change from medium free of toxin to medium with toxin was accomplished simply by closing and opening appropriate stopcocks in the reservoir system (Fig. 1b or 1c).

In certain experiments, the medium in the shell compartment was replaced with medium containing toxin (medium-change studies). In these studies, the perfusion through-the-bore of the capillaries was continued also with medium containing toxin, and the extracapillary change was carried out without stopping flow. The purpose of the medium change studies was to determine the minimum time at which an inhibition of oxygen utilization could be sensed.

In studies not involving rapid replacement of the extracapillary medium, diffusion of the toxin across the capillary walls (in through-the-bore introduction of toxin) and dilution of the toxin by the extracapillary medium occur initially. In through-the-shell perfusion of toxin, only the time for replacement of the shell volume with toxin-containing medium should delay the observable response.

In all studies, the oxygen utilization of the cells on the culture unit was measured for at least 30 min. Prior to this determination, the oxygen tension of the medium (both with and without toxin) was measured at the flow rate which was used to perfuse the capillary unit. The inlet medium oxygen tension for medium with or without toxin averaged 146 $\pm$ 7 mmHg (44 determinations, uncorrected for changes in barometric pressure). The outlet medium pO_2 from the culture unit was continuously measured by the oxygen electrode (IL) and recorded by a chart recorder. Silastic tubing (permeable to gases) was used to connect the reservoir to the unit to ensure that the medium was equilibrated with atmospheric oxygen. The unit outlet was connected directly to the oxygen meter flow cell. All measurements were made at 37°C.

4.1 Perfusion Through-the-Bore: Medium Change Studies

Toxicity studies have been performed in which the medium in the shell was replaced with medium containing toxin while, simultaneously, medium containing toxin was allowed to flow through the bore of the fibers. Since the oxygen content of the original shell medium is partially depleted at the time of change-over and the replacement medium is fully oxygenated, part of the initial increase in the exit medium oxygen content is due to equilibration of the capillary fluid with the oxygenated shell medium. This transient increase has been subtracted from the curves in Figures 2, 3, and 4 since this artifact would not be encountered in use. The amount of the apparent increase in exit oxygen tension due to this transient was obtained from the curves which result when the medium in the shell (of the same units used for the subsequent toxicity measurements) was replaced with medium not containing toxin; the replacement was performed in an identical manner for the control and the toxicity studies. Differences between such curves reflect the inhibition of oxygen utilization due to treatment with toxin (Figs. 2, 3, and 4). Correction for oxygen meter drift (downward) in Figures 2-4 is made by subtracting the comparable drift from medium flowing through the oxygen meter at the same flow rate. In Figure

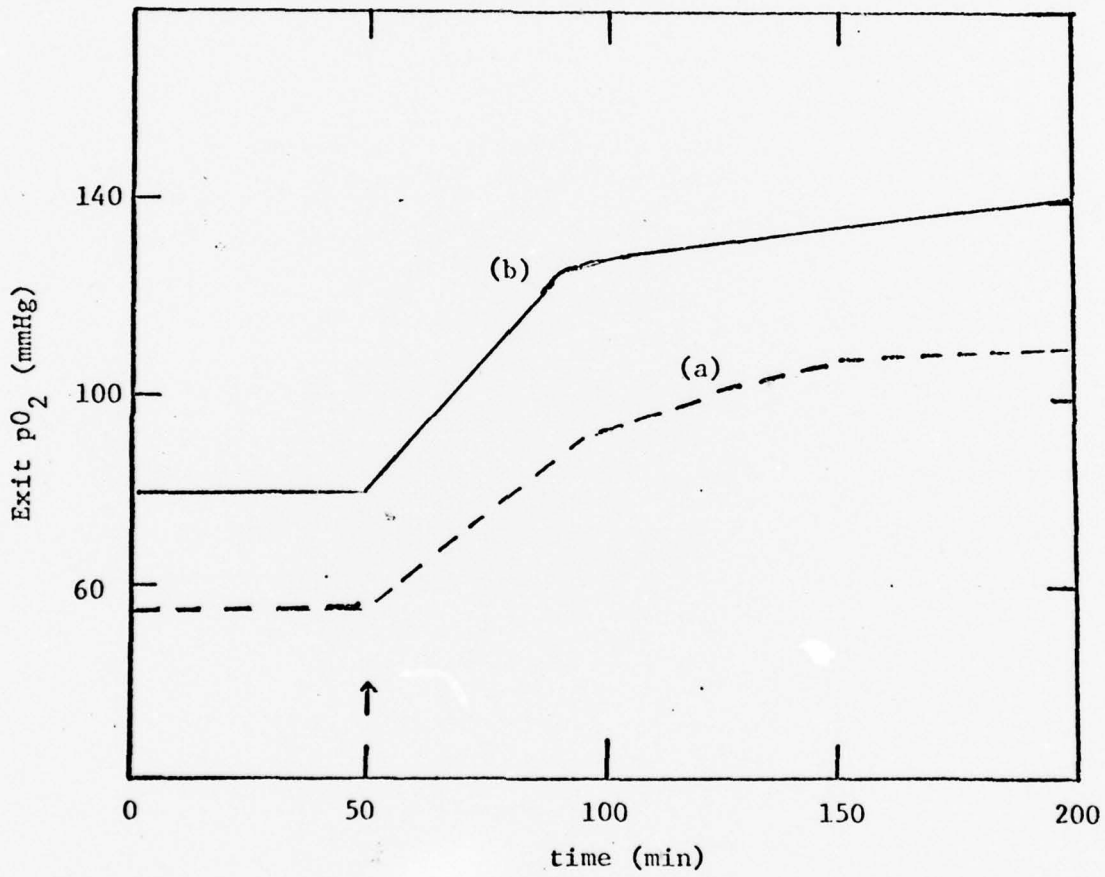


Figure 2. Effect of o-toluidine on oxygen utilization by cells on artificial capillaries (medium change studies).
Curve (a), L929 cells on an Amicon 3S100 unit were perfused with medium at a flow rate of 0.27 ml/min; at the arrow, the medium in the shell was replaced with fresh medium containing 28 ppm o-toluidine and perfusion with this medium was begun through-the-bore also.
Curve (b), BHK cells on an Amicon 3S100 unit were perfused at 0.25 ml/min. At the arrow, replacement of the extracapillary medium with medium containing 100ppm o-tol was performed. The curves have been corrected as described in the text.

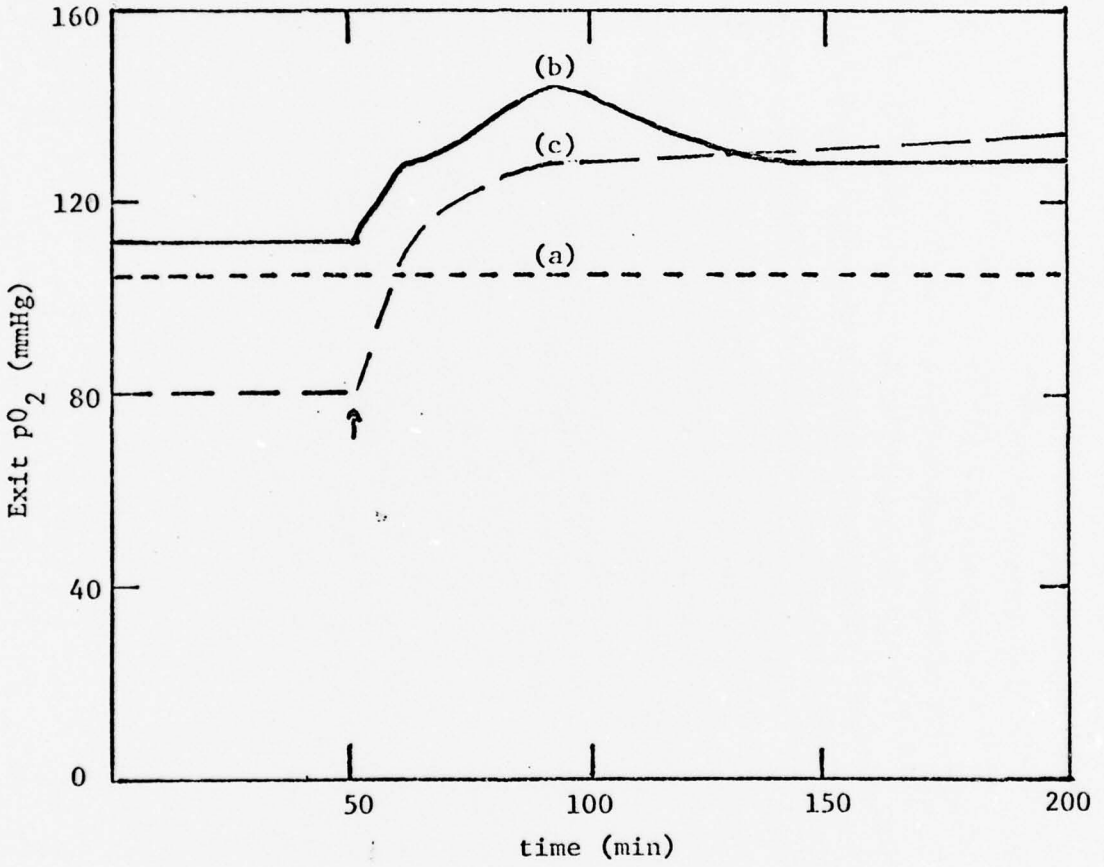


Figure 3. Effect of N,N-diethyl-m-toluamide on oxygen utilization by cells on artificial capillaries (medium change studies). L929 cells on Amicon 3x50 culture unit were perfused at 0.29 ml/min. At the arrow, the extracapillary medium was changed to medium with toxin and perfusion was begun through-the-bore with the medium containing toxin.

Curve (a) 10 ppm DET, (b) 50 ppm, (c) 100 ppm. The higher doses followed the lower doses on the same capillary culture unit. (Curves were corrected as described in the text.

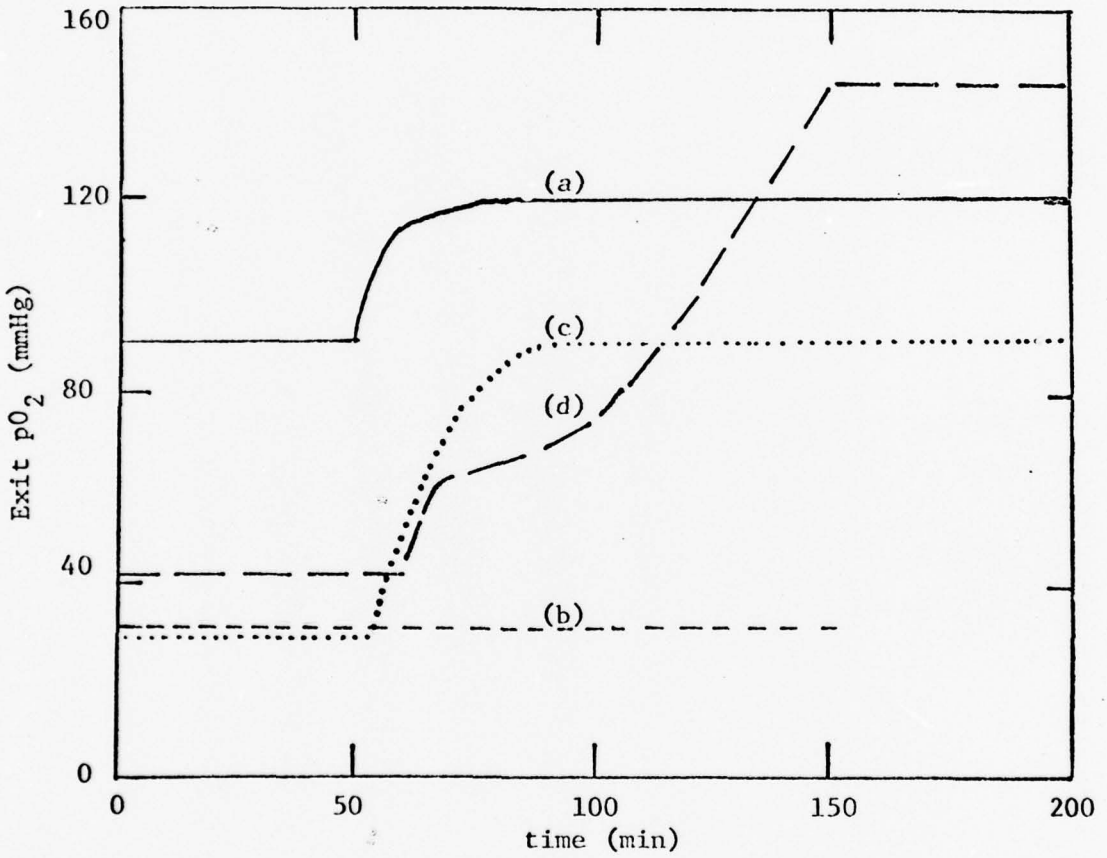


Figure 4. Effect of 2,3-dimethylphenol on oxygen utilization by cells on artificial capillaries (medium change studies).

Curve (a), L929 cells on an Amicon 3x50 unit, flow rate 0.16 ml/min, were treated as described in the legend to Figure 2 with medium containing 10 ppm 2,3 DMP.

Curves (b-d), L929 cells on GSRI-300 units, curve (c) with 100 ppm at a perfusion rate of 0.17 ml/min, curves (b,d) with 25, 100 ppm at a rate of 0.30 ml/min. All curves have been corrected as described in the text.

2, the effects of o-toluidine at 28 and 100 ppm (curves a and b) are shown using the medium change method of introducing medium containing o-toluidine; in Figure 3, the effects of N,N-diethyl-m-toluamide at 10, 50, 100 ppm (curves a-c) are shown; and in Figure 4, the effects of 2,3-dimethylphenol at 10, 25, 100, and 100 (curves a-d).

In certain experiments (diethyl-m-toluamide and dimethylphenol treatment), lower doses were followed by higher doses of the same toxin when inhibition of oxygen utilization did not occur at the lower dose. The curves for the 2,3-dimethylphenol toxicity studies (Fig. 4) have not been corrected for differences in the flow rates and culture units. In two studies, a 0.16 ml/min flow rate was used, and in two other studies, a rate of 0.31 ml/min was used. One of the studies was performed using an Amicon 3 x 50 culture unit (0.16 ml/min), and three studies with GSRI 300 fiber units. With the smaller Amicon unit, even at the slow flow rate, the toxicity of the 2,3-dimethylphenol is sensed sooner than when the GSRI 300 fiber units with their five-fold larger shell volume were used. The difference in flow rate affects the response time for the 2,3-DMP doses when the GSRI 300 fiber units were used. The greater number of cells on the unit using faster flow (curve d, Figure 4, initial oxygen utilization rate = 1.26 µl/min) compared to the unit using slower flow (curve c, Figure 4, initial oxygen utilization rate = 0.75 µl/min) caused a slight delay in the time at which the response reached a plateau. With this same type of unit (curve b, Fig. 4) no response was seen using 25 ppm DMP, although on an Amicon 3 x 50 unit a marked (and rapid) response occurred at 10 ppm. In the former study, the contact time of the cells with 25 ppm DMP must have been too short to permit detection of the toxin, whereas with the smaller extracapillary volume in the Amicon 3 x 50 unit, the toxicity was rapidly detected.

The percent inhibition of oxygen utilization (defined as the initial ΔpO_2 minus ΔpO_2 at time t, divided by the initial ΔpO_2) at 15, 30, 60 and 90 min after introduction of toxin is given in Table 11 for the three toxins studied by the medium change procedure. These values are calculated from the toxin response curves of Figures 3-5. The data indicate that these toxins partially inhibit oxygen utilization as early as 15 min after the initial treatment. In most cases, the amount of inhibition reaches a plateau by 60-90 min. The variance in these values is probably substantial; for example, for DMP at 100 ppm, the average of two determinations at 90 min is 65 $\pm$ 15% inhibition.

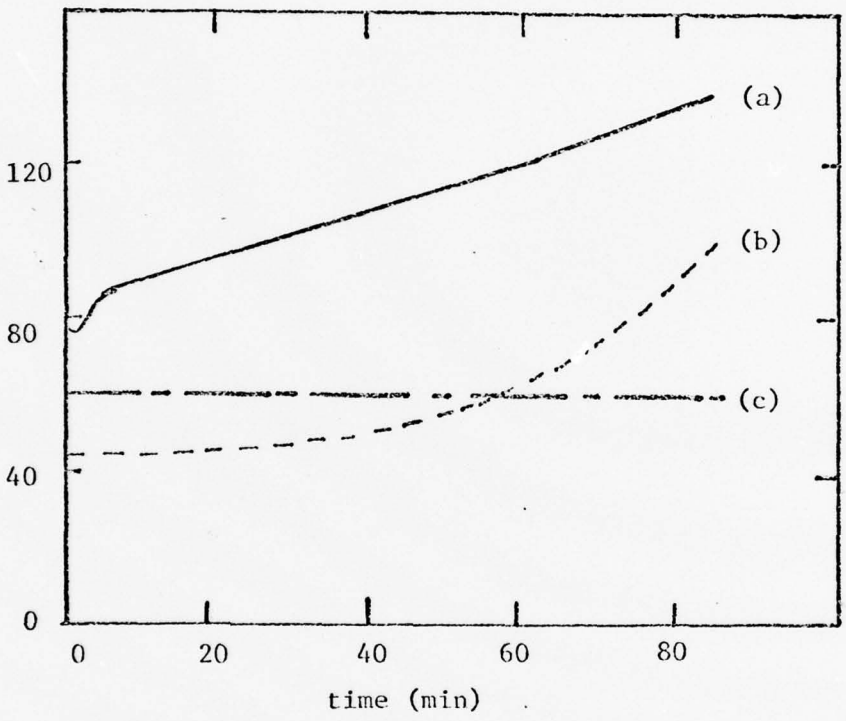


Figure 5. Effect of toxins on oxygen utilization: through-the-bore perfusion.

(a) Effect of 0.65 ppm potassium cyanide on BHK cells on an Amicon 3S100 unit, perfusion at 0.25 ml/min, starting time = 0.

(b) Effect of 100 ppm sodium iodoacetate on BHK cells on a GSRI-150 unit, perfusion at 0.25 ml/min.

(c) Effect of 100 ppm o-tol on BHK cells on a GSRI 150 unit perfusion at 0.25 ml/min.

TABLE 11. INHIBITION OF OXYGEN UTILIZATION: MEDIUM CHANGE STUDIES

Toxin	Concentration (ppm)	Percent Inhibition[a] After 15 min	30 min	60 min	90 min
N,N,DET	10	0	0	0	0
N,N,DET	50	53	68	68	47
N,N,DET	100	51	63	71	74
o-tol	28	13	27	44	54
o-tol	100	26	51	71	77
2,3-DMP	10	42	60	60	60
2,3-DMP	25	0	0	0	0
2,3-DMP	100 (1)	25	43	50	50
2,3-DMP	100 (2)	19	20	39	80

[a] $$\text{Percent inhibition} = \frac{(\text{outlet } pO_2)_t - (\text{outlet } pO_2)_o}{(\text{inlet } pO_2 - \text{outlet } pO_2)_o} \times 100$$

where t = time after introduction of toxin, 0 = time before introduction of toxin (inlet pO_2 is the pO_2 of the medium containing toxin).

The medium change method gives an indication of the minimum possible response time. All other methods for introducing toxin involve delays due to equilibration of the bore with shell medium (through-the-bore flow) or due to slow replacement of the toxin-free medium (through-the-shell flow). The results of the medium change studies indicate that these toxins can rapidly inhibit oxygen utilization by the cells on the capillary units. Further, the larger initial response with the Amicon units indicates that given the same cell number, a faster response may be achieved with a smaller shell holdup volume.

4.2 Perfusion Through-the-Bore or Through-the-Shell

The effects of various toxins on oxygen utilization by cells on artificial capillary units using either through-the-bore or through-the-shell perfusion have also been studied. In these studies, medium containing toxin was delivered to the capillaries by a peristaltic pump using the mode shown in Figure 1b; no separate step to replace the extracapillary medium was performed. All the toxins studied to date have been delivered to the cells either by this mode of perfusion or by the mode of Figure 1c in which the perfusion is through-the-shell.

Most of the experiments using either through-the-bore or through-the-shell perfusion were performed as follows. A two-reservoir system--one with toxin-containing medium, one with medium alone--was connected to the oxygen meter by ∿2 ft of Silastic tubing (0.0313 inch bore) (Fig. 1b, c). The medium contained 20 mM HEPES and 1% calf serum. The oxygen content of the medium (with or without toxin) was measured at 0.24 ml/min and a baseline established (or any drift recorded). The culture unit with established cells was then added to the perfusion system with toxin-free medium flowing. The exit pO_2 (from the bore or shell depending on the experiment) was recorded for at least 15 min before making a change to perfusion with medium containing toxin. From this procedure, the oxygen content of the medium entering the unit and that of the medium leaving the unit were determined using the same oxygen meter and the same flow rate. No significant difference in oxygen tension was found for medium containing toxin compared to medium without toxin. Flow rate was first estimated by setting the speed control dial of the peristaltic pump and later was measured directly on the medium leaving the oxygen meter.

The curves resulting from through-the-bore perfusion show the inhibition of cellular oxygen utilization as a function of time. Representative curves are given in Figure 5 for sodium iodoacetate (100 ppm, curve b), o-toluidine (100 ppm, curve c), and potassium cyanide (6.5 ppm curve a). Representative curves for through-the-shell perfusion of toxin are given in Fig. 6 for potassium cyanide (6.5 ppm, curve a), o-toluidine (100 ppm, curve b), sodium iodoacetate (100 ppm, curve c), 2,3-dimethylphenol (25 ppm, curve d), and hydroquinone (50 ppm, curve e). Using through-the-bore perfusion, little (or no) inhibition of oxygen utilization was sensed in short exposure times (less than 100 min) with o-toluidine (100 ppm); a rapid inhibition was found when the through-the-shell perfusion mode was used.

The percent inhibition of oxygen utilization at 15, 30, 60, and 90 min after introduction of toxin using through-the-shell perfusion is given in Table 12 for all the toxins studied to date. A comparison (on the same type of culture unit) between through-the-bore and through-the-shell modes of introducing toxin has also been made. These results are shown separately in Table 13. From Tables 12 and 13, it is clear that through-the-bore perfusion, in most cases, leads to a delay in the sensing of toxicity compared to perfusion through the shell. For o-tol, 2,3-DMP, phenol, and DNPH, this delay is beyond the 15-30 min sensing time desired for the MUST project. Furthermore, with

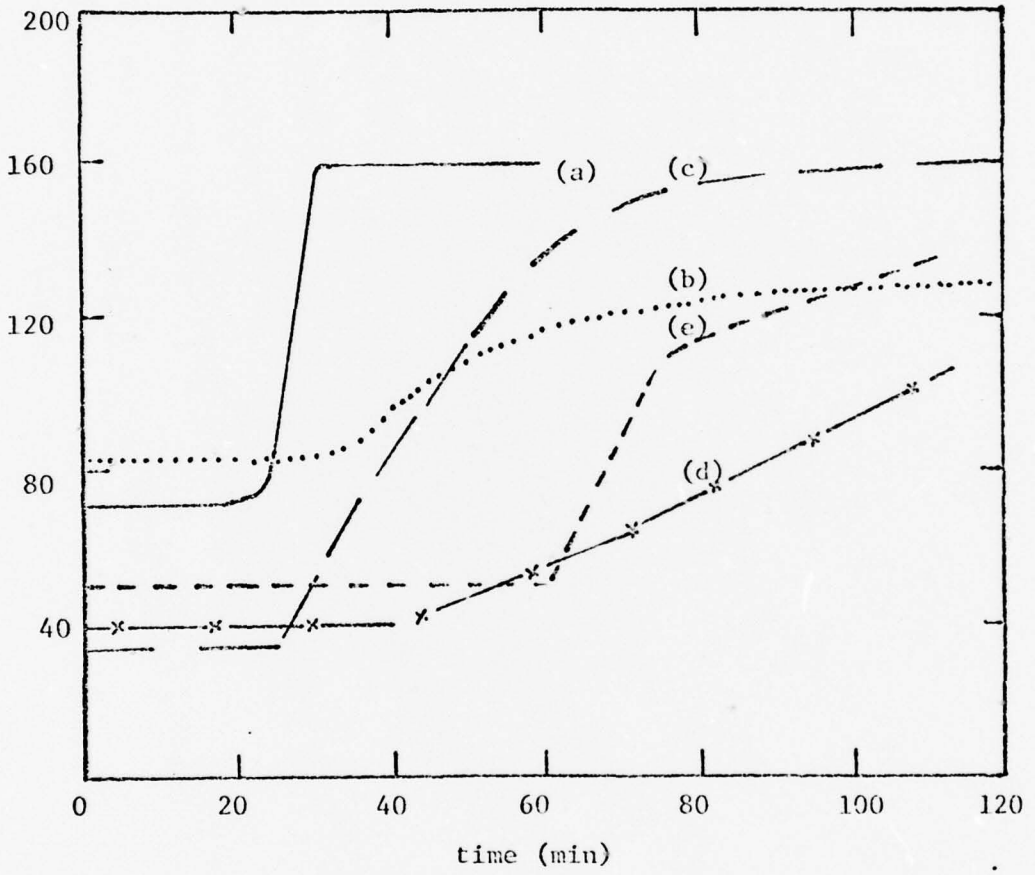


Figure 6. Effect of toxins on oxygen utilization: Through-the-shell perfusion. At t=20 min perfusion with medium containing toxin was begun.

(a) L929 cells on an Amicon 3S100 unit treated with 0.65 ppm potassium cyanide at a flow rate of 0.27 ml/min.

(b) BHK cells on an Amicon 3S100 unit treated with 100 ppm o-tol at 0.25 ml/min.

(c) BHK cells on a GSRI-150 culture unit treated with 100 ppm sodium iodoacetate at 0.25 ml/min.

(d) L929 cells on an Amicon 3x50 unit treated with 25 ppm 2,3-dimethylphenol at 0.26 ml/min.

(e) L929 cells on a GSRI-300 unit treated with 50 ppm hydroquinone at 0.34 ml/min.

TABLE 12. INHIBITION OF OXYGEN UTILIZATION: THROUGH-THE-SHELL PERFUSION STUDIES

Toxin	Concentration (ppm)	Flow Rate (ml/min)	Percent Inhibition After 15 min	30 min	60 min	90 min	Unit
o-tol	0.1	0.17	0	0	0	0	3x50
	1	0.31	0	0	0	NT[a]	3S100
	10	0.17	0	6	12	12	3x50
	10	0.31	4	8	8	8	3S100
	*10	0.17	0	0	0	0	3S100
	25	0.31	6	13	32	32	3S100
	28	0.27	8	100	NT	NT	3S100
	28	0.27	22	36	60	63	3S100
	50	0.31	6	16	38	44	3S100
	*50	0.17	0	0	0	0	3x50
	100	0.31	17	50	100	100	3S100
	100	0.25	6	39	62	71	3S100
2,3-DMP	1	0.26	0	0	0	0	GSRI-80
	*4	0.24	53	53	53	53	3x50
	10	0.26	0	3	11	11	3x50
	25	0.26	0	7	31	58	3x50
HQ	0.1	0.22	0	0	0	0	GSRI-80
	*1	0.21	64	64	64	64	GSRI-80
	1	0.31	7	11	NT	NT	3S100
	1	0.21	6	22	35	48	GSRI-80
	10	0.31	20	35	NT	NT	3S100
	*10	0.21	0	0	0	17	GSRI-80
	25	0.31	50	NT	NT	NT	3S100
	50	0.34	0	0	74	85	GSRI-300
DNPH	1	0.31	0	0	0	0	GSRI-80
	5	0.31	0	0	0	0	GSRI-80
	10	0.31	31	31	NT	NT	GSRI-80
	20	0.31	43	64	NT	NT	GSRI-80
DET	50	0.29	0	0	0	0	3x50
	100	0.29	0	0	7	16	3x50
	150	0.29	0	0	12	23	3x50
	500	0.29	0	0	12	63	3x50

[a]NT = not tested.

TABLE 13. EFFECT OF TOXINS ON OXYGEN UTILIZATION BY CELLS ON ARTIFICIAL CAPILLARIES COMPARISON OF THROUGH-THE-BORE AND THROUGH-THE-SHELL PERFUSION

Toxin	Concentration (ppm)	Flow Rate (ml/min)	15 min	30 min	60 min	90 min	120 min	960 min	Mode	Unit
o-tol	100	0.21	0	85	90	97	100	100	shell	GSRI-80
o-tol	100	0.24	0	1	1	4	9	25	bore	GSRI-80
2,3-DMP	100	0.24	2	54	71	96	96	100	shell	GSRI-80
2,3-DMP	100	0.24	1	1	1	2	4	10	bore	GSRI-80
HQ	50	0.20	2	42	62	76	96	100	shell	3x50
HQ	50	0.23	15	26	34	41	46	95	bore	3x50
KCN	10	0.24	42	100	100	100	100	100	shell	GSRI-80
KCN	10	0.24	40	100	100	100	100	100	bore	GSRI-80
phenol	100	0.21	0	0	1	5	14	76	shell	3x50
phenol	100	0.21	0	0	0	0	0	9	bore	3x50
DNPH	satd	0.24	0	8	18	31	41	100	shell	GSRI-80
DNPH	satd	0.24	0	0	1	4	12	41	bore	GSRI-80
KCN	0.65	0.25	100	100	100	100	NT[a]	NT	shell	GSRI-150
KCN	0.65	0.25	21	33	58	92	NT	NT	bore	GSRI-150
IAc	100	0.25	33	69	100	100	NT	NT	shell	GSRI-150
IAc	100	0.25	0	3	17	62	NT	NT	bore	GSRI-150

[a]NT = not tested.

phenol, o-tol, and 2,3-DMP, the inhibition is still less after 960 min than when through-the-shell perfusion was used with the same doses of these toxins, indicating that some phenols may be absorbed by the fiber polymer.

In certain of the experiments listed in Table 13, the inhibition of oxygen utilization is far from values anticipated on the basis of other data using the same toxin. These data, which have been marked with an asterisk in the table, are excluded from the dose-response curves (for through-the-shell perfusion) in Figures 7-10. In studies using concentrations ranging from 10-100 ppm, o-toluidine was found to partly inhibit cellular oxygen utilization within 90 min. In two experiments, one with 10 and another with 50 ppm o-tol, no inhibition was found. With hydroquinone (at 1 ppm) and 2,3-dimethylphenol (at 4 ppm), large, rapid inhibition of oxygen utilization occurred soon after introduction of toxin. The early inhibition (at 15 min) did not increase with time as was found for other doses of these toxins and with the other toxins studied. Furthermore, these doses were more toxic than expected from other data given for the same toxins. We suspect that these results are artifacts and do not represent toxicity to L929 or BHK. Such artifacts could arise from several sources, including unrecognized microbial contamination of the culture unit; presence of trapped air bubbles in the oxygen meter electrode chamber; sudden change in flow rate of the medium stream; change in temperature of the perfusing medium, and loss of metabolizing cells from the culture unit shell.

To minimize such artifacts, each unit was routinely tested for microbial contamination by culture of an aliquot in Brain Heart Infusion broth and thioglycollate medium for 24 hr. However, contamination could occur after the aliquot was taken. Units with cloudy medium either in the shell or reservoir were not used, even if the tests for microbial contamination were negative. Contamination could lead to a lack of response to toxin if the contaminant is insensitive to such concentrations. The presence of air in the chamber of the electrode would lead to a sudden rise in the medium pO_2. Upon switching from medium without toxin to medium with toxin, the system was checked to make sure no bubbles were trapped in the electrode chamber. It is possible, however, that the increases in oxygen utilization in the suspect experiments with 2,3-DMP and HQ arose from this cause. The other possible causes of artifacts (change in temperature or flow rate) are less likely. The oxygen monitoring is sensitive to temperature changes, but the medium was always preequilibrated to 37°, and the warm room in which

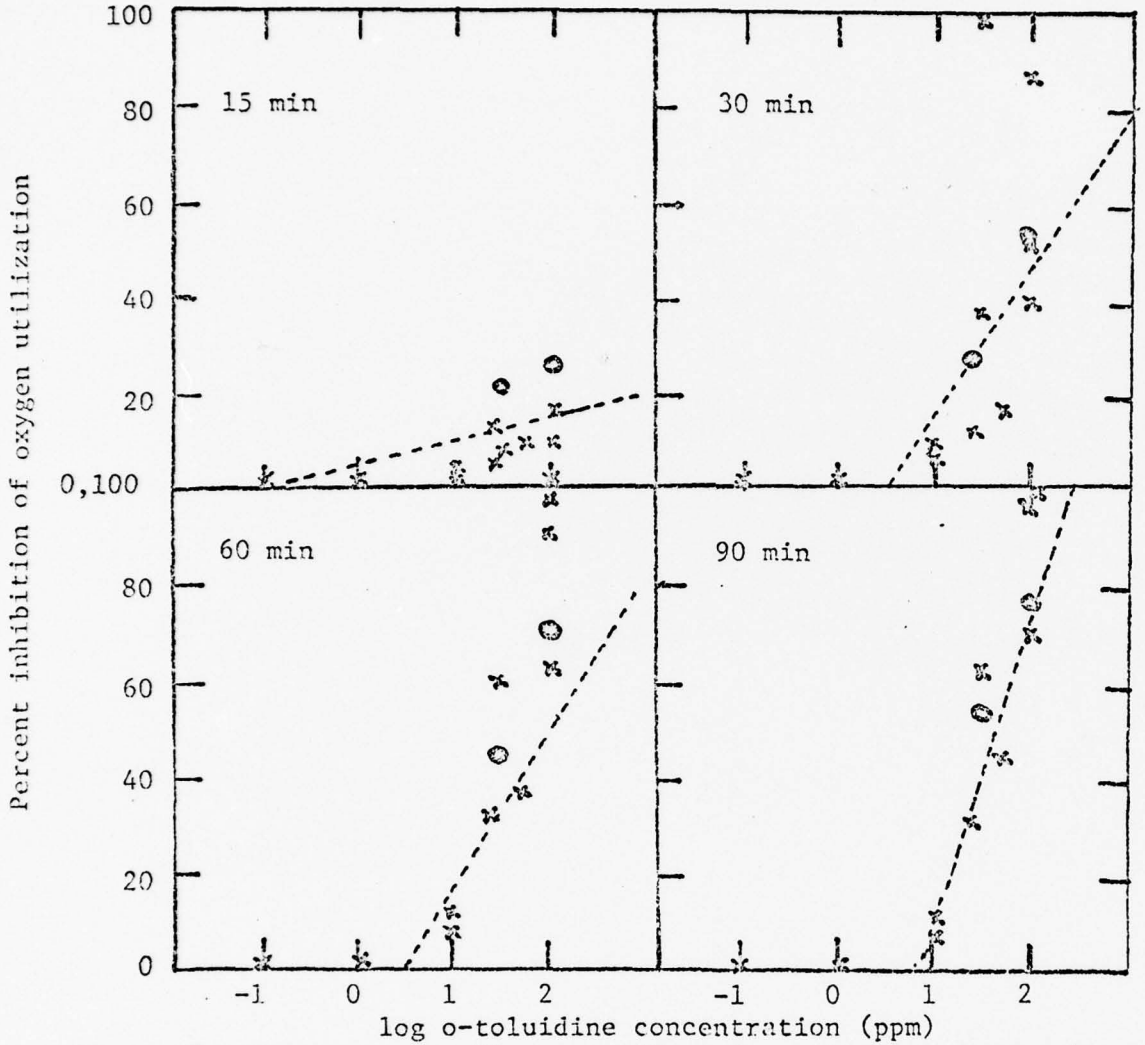


Figure 7. Response of cells on artificial capillaries to varying concentrations of o-toluidine change studies. Crosses - results from through-the-shell studies. Circles - results from medium change studies.

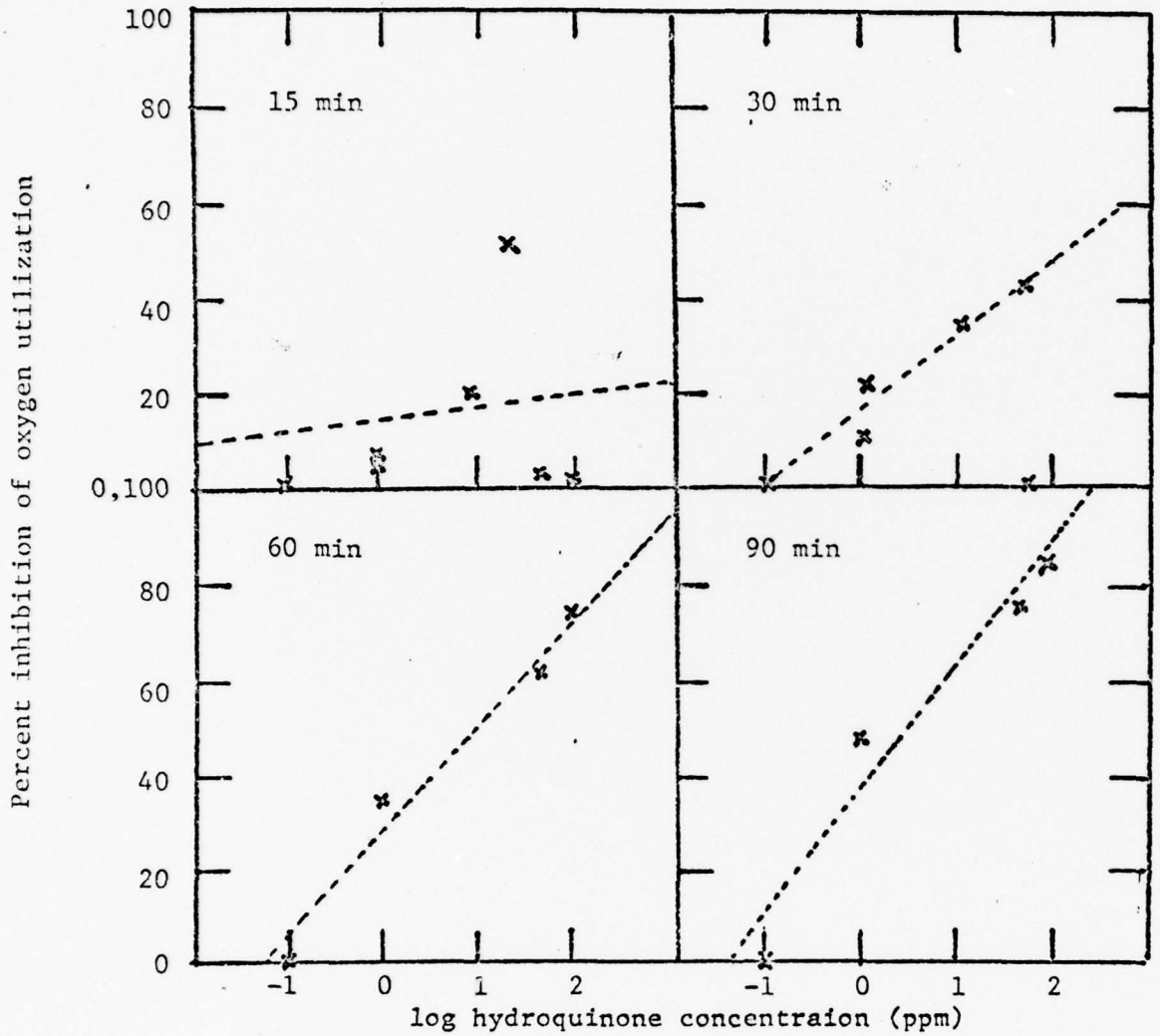


Figure 8. Response of cells on artificial capillaries to varying concentrations of hydroquinone.

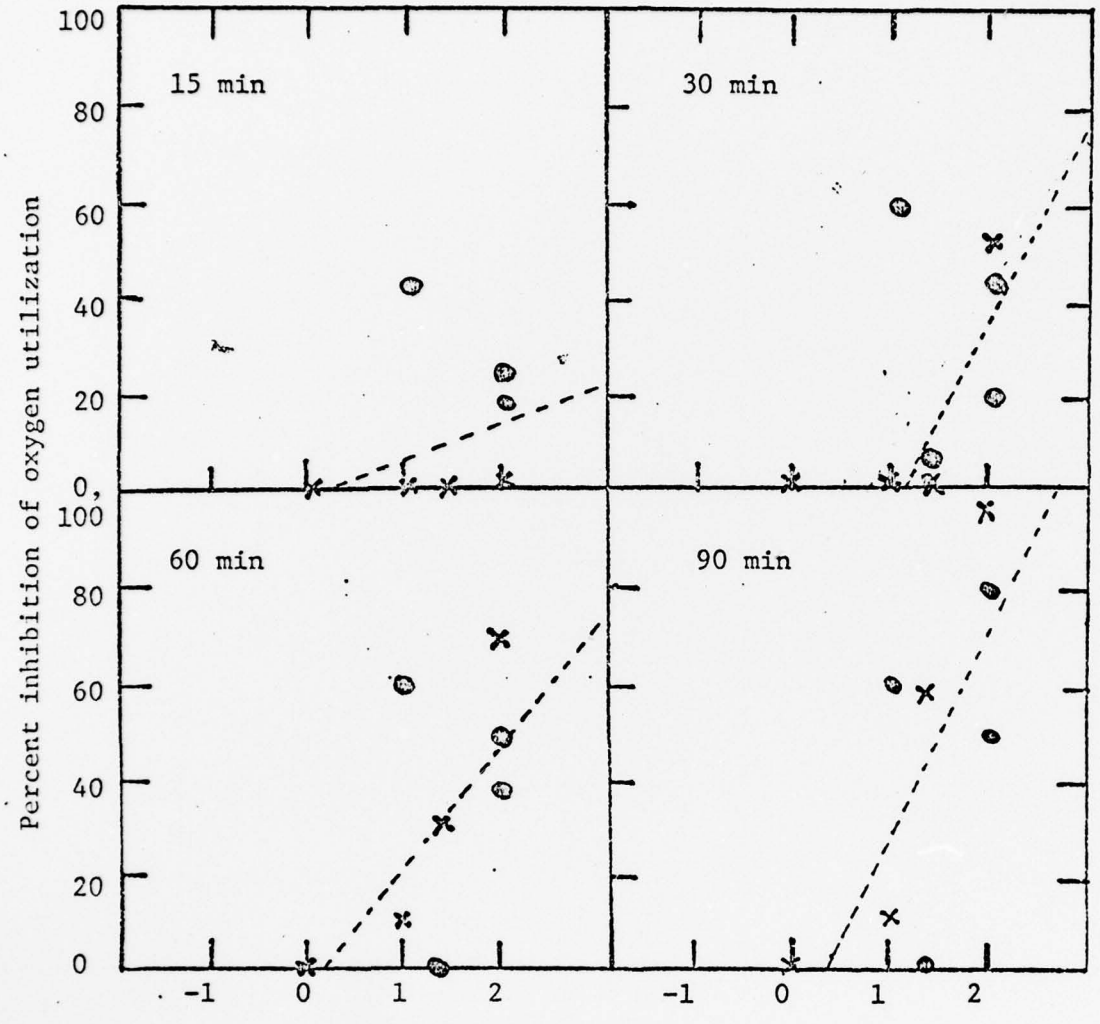


Figure 9. Response of cells on artificial capillaries to varying concentrations of 2,3-dimethylphenol change studies. Crosses - results from through-the-shell studies. Circles - results from medium change studies.

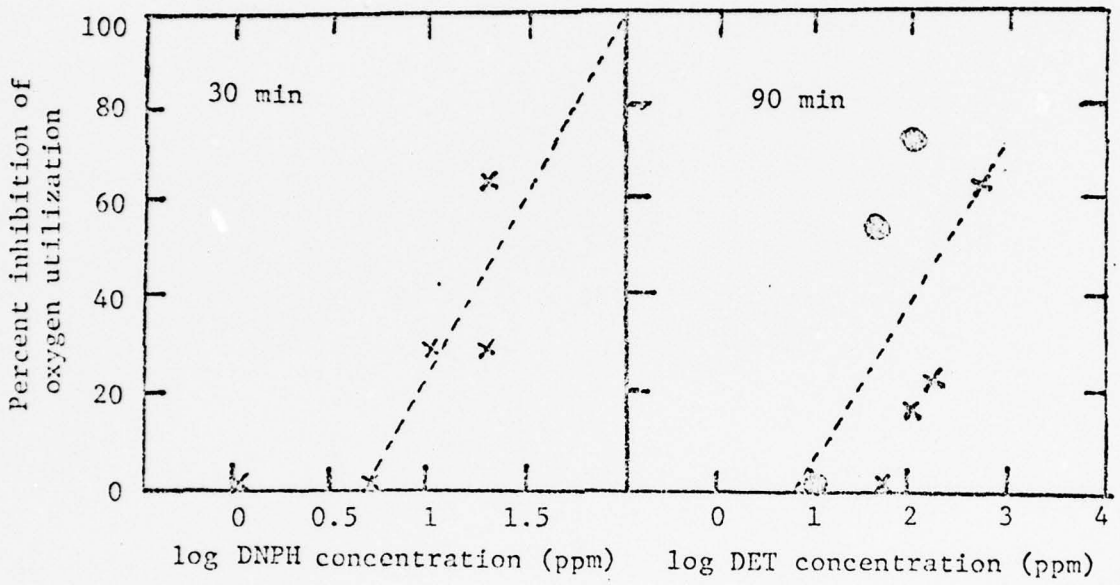


Figure 10. Response of cells on artificial capillaries to varying concentrations of 2,4-Dinitrophenylhydrazine and N,N-Diethyl-m-toluamide change studies. Crosses - results from through-the-shell studies. Circles - results from medium change studies.

these experiments were carried out is large enough that small fluctuations in temperature would occur only slowly. The oxygen meter reading has been found to be flow-rate dependent only when the electrode membrane is dirty, broken, or microbially contaminated. These occurrences would also lead to a severe drift in readings with time and a loss of calibration, both of which are checked before beginning an experiment. Loss of cells from the unit shell could lead to a decrease in oxygen utilization. We have found, however, that the live cells adhere strongly to the fibers (and wall of the unit) and are not easily washed out. Furthermore, nontoxic medium was passed over the cells (through-the-shell) for at least 15-30 min before the toxic medium was introduced. A visual check was made,however, to see if cells were present in the effluent after making the change from medium free of toxin to medium containing toxin.

Dose-response curves for each toxin can be plotted from the data given in Tables 11, 12 and 13. These are given for the toxins o-tol, HQ and 2,3-DMP in Figures 7, 8, and 9, and for 2,4-DNPH and DET in Figure 10. The data from both medium change and through-the-shell perfusion studies have been used. A graph of the percent inhibition of oxygen utilization versus log of the concentration of toxin (ppm) at different times after beginning the perfusion of toxin-containing medium is in Figures 7-9. Early in the experiments (15, 30 min), the response to toxin was less than at later times (60, 90 min), and the scatter in the points was greater. The curves indicate that toxicity can be rapidly detected with most of the toxins studied, but that the slopes of the dose-response curves are less than at later times. The toxicities at different doses have been compared, in most cases, for cells treated on different units and at different flow rates. Changes in these operating parameters will affect the immediate response detected more than the response at later times. However, by 90 min, the maximum percent inhibition at a given dose probably has not been reached in most of the experiments. Therefore, since different culture conditions were used (flow rate, type of unit), it is not surprising that a large variability is found in the data. We are attempting to minimize such variability in future experiments by using identical units for all doses of toxin and keeping the flow rate constant.

From the dose-response curves obtained by least-squres fit of the points, an EC_{50} value (concentration which causes 50% reduction in oxygen metabolism

of cells on artificial capillaries) can be computed for all toxins studied in this way. The statistical data presented in Table 14 indicate how well the points fit the straight line. In Table 15, the EC_{50} values calculated from the line are compared for the different times after subjecting the cells to toxin. They are compared in Table 16 to the LC_{50} values (concentrations which causes 50% reduction of protein synthesis in cell culture after 72 hr of continuous treatment) obtained by Christian et al.[8,9] for the same toxins. As can be seen in Table 14, the EC_{50} values at 15 min are completely unrealistic; however, those for 30, 60, and 90 min are in reasonable agreement. As the time of measurement increases, the EC_{50} values approach the LC_{50} values (Table 16), indicating that inhibition of oxygen utilization parallels cellular growth inhibition. Corroboration of these results is needed to assess the reliability of the artificial capillary toxicity detector for sensing these and other classes of toxins. It is possible that certain toxins would have no effect on cellular oxygen metabolism or might cause an increase in cellular respiration. Others could be inactivated by the cells before exerting their toxicity. These questions must be resolved before oxygen metabolism of cells on artificial capillaries can be assumed to be a generally applicable method for determining the toxicity of a water stream.

4.3 Reversibility of the Toxic Response

In several studies, we tested the ability of the cells to recover from a treatment with toxin. Recovery was measured by the restoration of a pretreatment oxygen utilization rate following change of toxin-containing medium to medium free of toxin. Table 17 lists the doses, times of treatments, and time for recovery for the toxins studied in this way. In some cases, multiple doses were given to the same cell culture units, and recovery was assessed after each dose. These data are limited, but indicate that short treatments with toxin do not sufficiently harm the cells to prevent relatively rapid recovery; prolonged treatment does cause sufficient cell damage to prevent recovery (even after 24 hr).

TABLE 14. LINEAR REGRESSION ANALYSIS OF DOSE-RESPONSE PLOTS FOR VARIOUS TOXINS

Toxin	Time	Slope	Standard Deviation	Coefficient of Determination
DNPH	30	75.1512	36.6853	0.677
DET	90	33.7803	21.3204	0.334
DMP	15	16.9810	10.7038	0.456
DMP	30	41.7515	14.7719	0.666
DMP	60	26.2726	22.9335	0.208
DMP	90	44.3180	27.4034	0.343
o-tol	15	7.9735	6.7187	0.135
o-tol	30	31.6976	23.9399	0.180
o-tol	60	39.5029	21.6004	0.295
o-tol	90	73.3597	13.7781	0.780
HQ	15	1.9141	11.0176	0.007
HQ	30	15.8488	2.2535	0.943
HQ	60	22.5093	3.3544	0.957
HQ	90	25.8426	4.6984	0.938

TABLE 15. EC_{50}[a] VALUES FOR VARIOUS TOXINS

Toxin	Time after Initial Exposure to Toxin 15 min	30 min	60 min	90 min
o-tol	$>10^6$	100	63	32
HQ	$>10^5$	110	10	3.2
DMP	$>10^4$	120	103	36
DNPH	b	22	b	b
DET	b	b	b	250

[a] EC_{50} = concentration which causes 50% inhibition of oxygen utilization by cells on artificial capillaries.

[b] Dose response curve not plotted

TABLE 16. COMPARISON OF CONCENTRATIONS OF COMPOUNDS WHICH CAUSE 50% REDUCTION IN PROTEIN SYNTHESIS IN CELL CULTURE (LC_{50}) AND 50% REDUCTION IN OXYGEN METABOLISM OF CELLS ON ARTIFICIAL CAPILLARY UNITS (EC_{50}) AT 90 MIN

Compound	LC_{50} (mg/1)	EC_{50} (mg/1)
Hydroquinone	0.8	3.2[a]
2,4-dinitrophenylhydrazine	4.5	22
2,3-dimethylphenol	18	36
N,N-diethyl-m-toluamide	119	250
o-toluidine	26	32

[a] EC_{50} measured at 30 min.

TABLE 17. REVERSIBILITY OF TOXIC RESPONSE

Toxin	Concentration (ppm)	Length of Time for Treatment (min)	Length of Time for Recovery (min)	Flow Rate (ml/min)	Unit
KCN	10	3	147	0.31	3S100
HQ	1	40	50	0.31	3S100
HQ	10	40	50	0.31	3S100
HQ	25	30	165	0.31	3S100
HQ	50	160	No Recovery	0.34	GSRI-300
DNPH	20	20	30	0.31	GSRI-80
DNPH	10	30	20	0.31	GSRI-80
o-tol	28	30	70	0.27	3S100

5. DEVELOPMENT OF A PROTOTYPE BIOLOGICAL DETECTOR

The data from culture of cells on artificial capillaries and from the oxygen inhibition studies show that the present perfusion system is suitable for a prototype toxicity detector. A scheme for such a prototype is shown in Figure 11. In this scheme, medium at twice normal concentration is fed through the same peristaltic pump as the water stream to be tested. Flow is regulated by meters and valves and is then mixed using a standard mixing coil such as that used in autoanalyzers. After mixing, the medium stream at normal strength is fed through the oxygen electrode compartment.

Side and top views of the electrode chamber are shown in Figure 12. The chamber, made from polypropylene or polycarbonate, is autoclaved before use. The entry and bypass channels could be machined into the plastic electrode chamber. The chamber is formed by gluing together identical halves with appropriate channels cut out to form the electrode well. The chamber must be designed so that holdup and liquid volume are minimal and bubble entrapment does not occur. The electrode can be sterilized by immersion in benzalkonium chloride followed by washing in sterile distilled water. A suitable oxygen microelectrode, such as that from Instrument Laboratories, is used to record the oxygen tension in this inlet stream. The separate entry and exit tubes into the electrode chamber are necessary to calibrate the electrode by introduction of the calibrating gases. A bypass tube is included so that medium flow to the cells will continue during the calibration of the electrode.

After passage through the first electrode, the medium enters the culture unit. Entry to the shell or the fiber bore or both is made by opening the appropriate stopcocks. An identical electrode at the exit measures the oxygen tension of the outflow medium. The picoammeter, which senses electrode response, measures the differential current of the two electrodes or (by means of a switching mechanism) the current of either electrode separately; the medium then goes to waste.

For the prototype, all valves are manually operated. The electrodes and the culture unit are maintained at 37°C either by a heating block specially machined to fit each component or, more simply, by immersion in a water bath. The culture units currently in use would require a heating

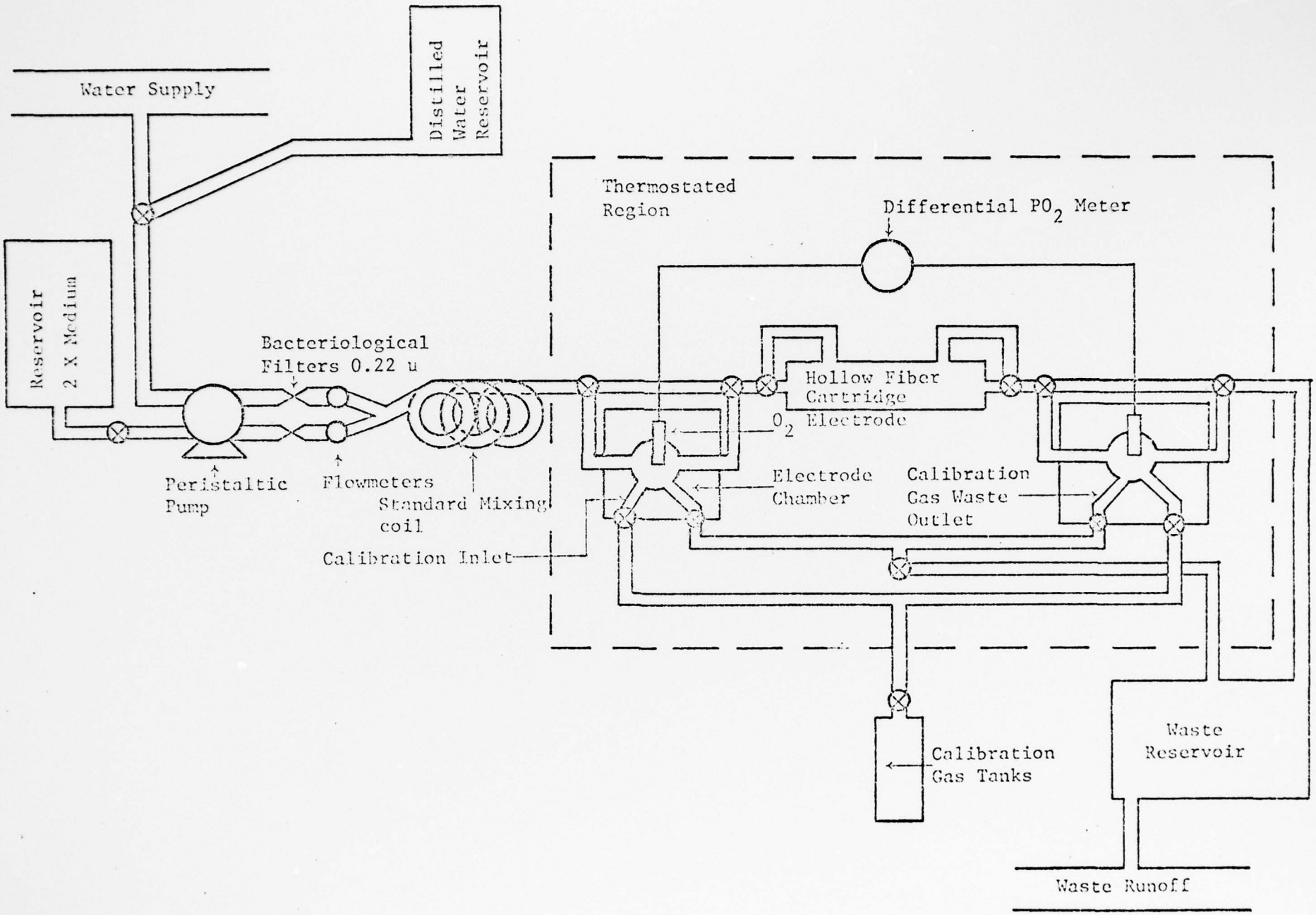
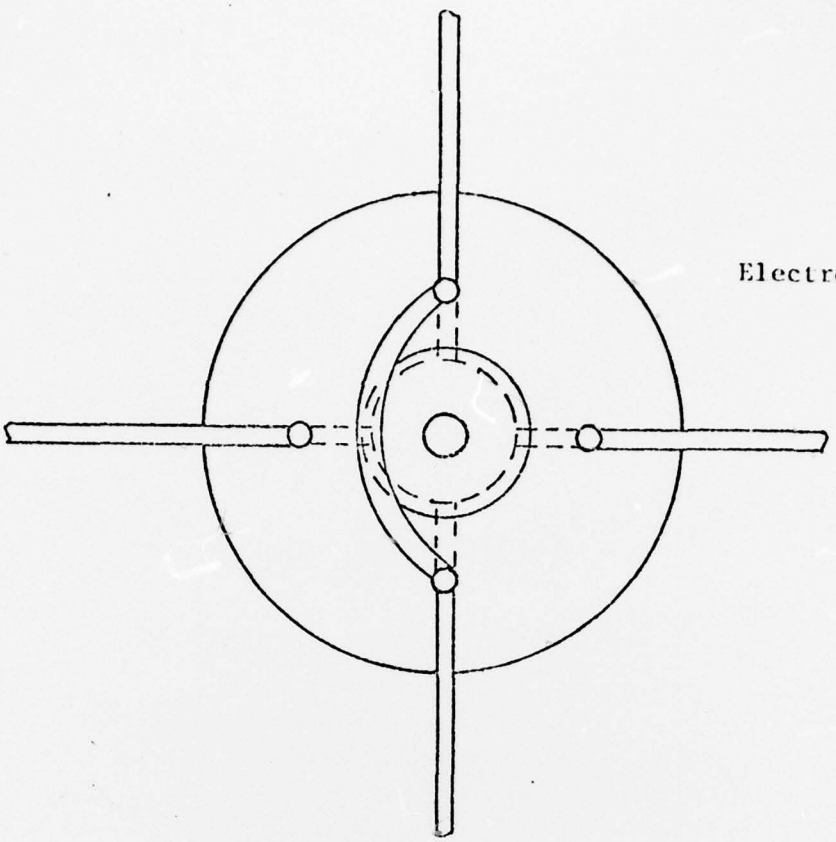
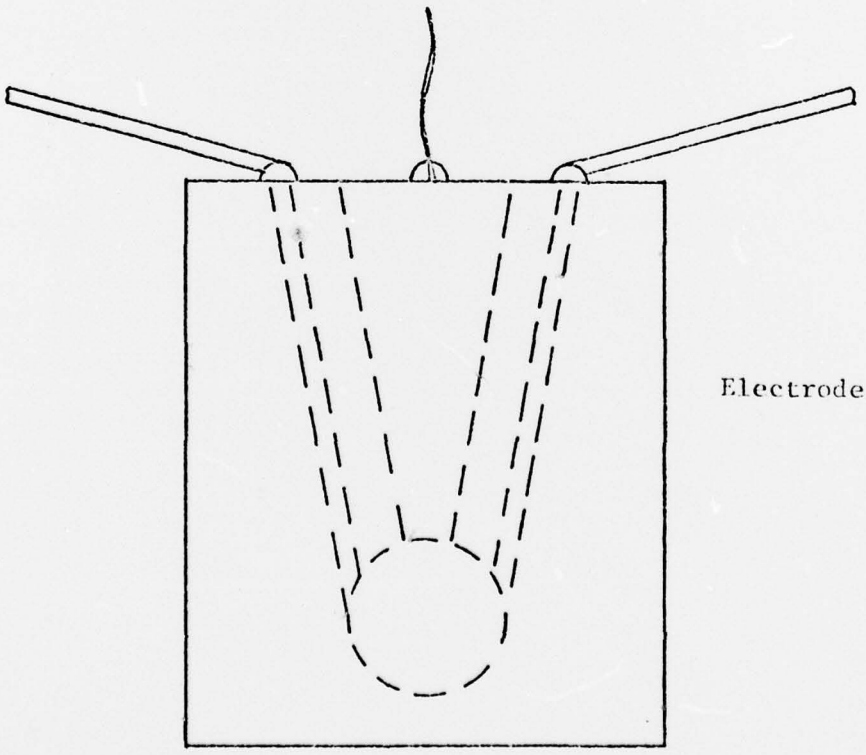


Figure 11. Prototype Detector



Electrode, top view



Electrode, side view

Figure 12. Electrode Chamber

block length of at least 12 cm; the electrode compartments such as that shown in Figure 12 could be designed to require no more than 2 cm apiece in such a dry block heater. Therefore, a minimum heating block length of 18-20 cm would be necessary. A stainless steel coil of tubing embedded in the block must be long enough so that temperature equilibration would occur before the medium reaches the first electrode. At the anticipated flow rates necessary to maintain the units (0.1-0.3 ml/min), this length can be as short as 30 cm.

The length of tubing leading from the reservoirs to the culture unit would be kept to a minimum to avoid delay in the detector response time. The prototype described here does not require medium preequilibrated with oxygen if 0.3-0.5 m Silastic tubing is used to connect the reservoir with the culture unit. Fittings leading directly into the unit are of a quick disconnect type, such as Luer, so that culture unit replacement can be readily made under sterile conditions.

For each thermostated block or water bath, two capillary culture units are maintained; one for monitoring purposes and one to be maintained under identical conditions to serve as a replacement. The second does not have adaptation for oxygen metering. Other replacement units, also without oxygen metering, could be maintained with a similar thermostated block. A water bath (Thelco Model 82 from Precision Scientific Company) is currently used to maintain the 37°C temperature necessary for both the oxygen electrode and for the artificial capillary unit. The culture unit can be completely immersed in the water bath to provide precise temperature control. An IL electrode fitted into a flow-through cuvette made from polycarbonate is being used to measure outlet pO_2. The electrode chamber is maintained at 37°C by an external circulating water bath.

The electrode and culture unit are in-line with a reservoir held at 22°C. Connection of the reservoir with the culture unit was made with 70 cm of Tygon tubing to connect the stainless steel tubing with the unit. Fittings are 17-gauge Luer-lok (female) needles (stainless steel) adapted to stainless steel Luer-lok male connectors (Extracorporeal).

This setup was tested to determine if it would provide warm (37°C) medium to both the culture unit and the electrode. A YSI (Yellow Springs Instruments) Model 44TC Tele-Thermometer with a banjo-type thermistor probe was used for these measurements. The probe was inserted into a piece of

amber rubber tubing, which was connected by a short piece of Tygon tubing (6 cm) to the steel coil described above. Equilibrium temperature was measured with the thermistor probe above the water bath (air temperature of 28°C) and with the probe out of the water bath. Water from a reservoir was pumped through the tubing and past the probe at two different flow rates. The temperatures recorded by the probe maintained under these different conditions are given in Table 18. As shown, at a 2 ml/min flow rate, water at room temperature is warmed to 37°C after passing through the metal coil heat exchanger. At a flow rate of 5 ml/min (the fastest possible with this tubing), the medium may not have reached 37° by the time its temperature was sensed by the thermistor. The thermistor comes to equilibrium quite rapidly (1-2 sec); therefore, these numbers should reflect accurately the actual temperature of the entry water. With the thermistor probe out of the water bath, slight cooling due to re-equilibration with the ambient air temperature was noticeable at flow rates less than 1 ml/min. The temperature of the water leaving the steel tubing, however, has more time to equilibrate with the bath temperature at slower flow rates. Since the culture unit system uses a flow rate of ∿0.2 ml/min rather than 2 ml/min, the temperature of the medium will equilibrate with the bath temperature before reaching the culture unit.

These results indicate that an in-line metal coil will provide adequate thermal equilibration to warm the room temperature medium to the 37° temperature for tissue culture. The same coil can also serve to mix the flows from the water stream (containing toxin) and culture medium at 2X final strength.

The system was tested to determine if mixing of the two solutions was adequate. Two reservoirs were used for this experiment; the first with water and the second with a dilute Trypan Blue solution (A_{580} = 0.348 nm). Upon mixing the solutions 50:50, the A_{580} was 0.152 nm. Table 19 shows the A_{580} of the effluent mixed by the stainless steel coil described above. The mixing was assayed at different flow rates. As can be seen in Table 19, the saline solution was in slightly greater concentration at high flow rates (5.4 ml/min) than at low flow rates. No convection currents were visible in the sample, indicating that the solution was homogeneous. These results show that simple mixing of two streams is relatively straight-forward at the flow

TABLE 18. TEMPERATURE CONTROL USING A 37°C WATER BATH

$T_{reservoir}$	T_{bath}	Flow Rate (ml/min)	T Thermistor in water, coil in water	T Thermistor out of water, coil in water	T Thermistor out of water, coil out of water	T Thermistor in water bath, coil out
20	37	0	37°	28	28	37
20	37	2	37°	37	22	30
20	37	5	37°	35	20	28

TABLE 19. EFFECT OF FLOW RATE ON MIXING OF TWO STREAMS

Flow Rate (ml/min)	A_{580}
5.4	0.140
4.4	0.145
4.1	0.146
3.8	0.150
3.4	0.152
2.8	0.158
1.2	0.152
0.6	0.151

rates which will be used and with a multiple channel peristaltic pump whose flow rate is the same for each channel. Such pumps are available from Brinkmann Instruments and Buchler.

The prototype detector described above is suitable for cell culture and toxicity studies outside the warm room. This should allow such studies to be performed more conveniently and should demonstrate the adaptability of artificial capillary cell culture to a relatively mobile, simple apparatus suitable for the ultimate requirements of the MUST program.

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